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Memento Mori: Investigating Mummies for Ancient Diseases

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Terezia Hausmann lived during the Austro-Hungarian Empire, in the town of Vác, Hungary. She died aged 28 on December 25, 1797. Hausmann's naturally mummified body, along with that of 241 others, was found in 1994 in two walled-off crypts under a Dominican church undergoing renovations. Two-thirds of the bodies had become naturally mummified, preserved with their fine wool and lace clothing, and wooden coffins painted with inscriptions like "memento mori."

Hausmann died 4 years after her mother and less than 3 years after her sister, likely from tuberculosis (TB). "Both the daughters were incredibly wasted," says Helen Donoghue, Ph.D., and honorary senior lecturer in the Division of Biosciences at the Centre for The History of Medicine and Division of Infection and Immunity, University College London (UCL). "The younger daughter, Barbara Hausmann, died at age 14. When we found her body, my collaborator, a doctor, thought she might be about 8 years old."

Prior PCR had confirmed TB, but in 2004, researchers used metagenomic analysis—the open-ended sequencing of DNA recovered from uncultured samples without target-specific amplification or enrichment—to sequence DNA from Terezia Hausmann's lung tissue. They concluded that she was coinfected with two different strains of *Mycobacterium tuberculosis* (MTB). The researchers used patterns of deletion of genetic loci and 300 shared genetic variants to link the two strains of MTB found in the mummy to an outbreak that occurred in Germany from 1998 to 2010 (Chan et al., 2013).

Donoghue is a member of an eclectic community of scientists, physicians, and adventurers who investigate ancient infectious diseases such as plague, smallpox, typhus, and influenza. They use tools like CT scanners, PCR, bioinformatics, and whole genome sequencing to solve historical mysteries that could have bearing on modern diseases.

The Granville Mummy, Revisited

In 1825, Dr. Augustus Bozzi Granville, a gynecologist, conducted the first scientific mummy autopsy on an Egyptian aristocrat in her 50s named Irtyersenu. He presented his conclusion that she had died of an ovarian tumor to the Royal Society. In 2009, Donoghue and her collaborator, Dr. Mark Spigelman at UCL, conducted PCR on tissue samples and determined the tumor was benign: Irtyensenu had died of disseminated TB.

"A lot of diseases we attribute to modern times are probably more ancient than we think."— Dr. Frank Rühli

MTB has been around for a long time. "We found it in Pleistocene bison 17,000 years ago," says Donoghue. "And we found it in 9000-year-old Neolithic settlements in the eastern Mediterranean." MTB doesn't have an environmental reservoir. "If you look at different strains, they have a clonal relationship," says Donoghue. "It just looks as though the pathogenic bacteria and the human hosts have coevolved." According to Donoghue, this suggests that someone living in San Francisco or in the Indian subcontinent would be more than likely to become ill with the strain that coevolved with them, but familiarity over millennia can make the disease slower to kill.

"*Mycobacteria tuberculosis* evolve by losing bits of their DNA," says Donoghue. "You are looking at deletions and sometimes internal rearrangements. You don't get horizontal gene transfer, not since they have become obligate pathogens." The World Health Organization estimates that today a third of the global population is infected with TB, but almost all of it is latent. Only 10% or so is liable to develop active infection; TB led to an estimated 1.4 million deaths in 2011. According to Donoghue, this level of latency can be attributed more to nature of the host than the immune response or resistance to infection. Latency, however, may be also related to the lineage of the strain.

The WHO estimates that about 3.7% of newly diagnosed TB patients have multidrug-resistant (MDR) TB, and the levels in previously treated patients are about 20% higher. About 9% of MDR-TB patients have extensively drug-resistant (XDR) TB. By March 2013, 84 countries reported at least one XDR-TB case.

Some of Donoghue's clinical colleagues in London are now talking about reviving old methods of treating TB, such as the lung-collapsing surgery performed before antibiotics were invented. "That's why I think it is important for people like me and other people in the field, what we call paleomicrobiologists, to examine the relationship between *Mycobacterium tuberculosis* and its hosts in the era before there were any antibiotics where the only resistance is the natural resistance a person might have due to nutrition, background, and genetics," says Donoghue.

These days, instead of relying on historical records and bone lesions to diagnose TB in mummies, researchers use a technique called "size-gating" to sort out potential contamination, eliminating DNA larger than a certain number of base pairs, because all modern DNA will be larger. Whole genome sequencing of mummy tissue is commonly done using "bait," a microarray or a magnetic beam coated with a single-stranded version of modern DNA, which is used to fish out any ancient DNA that matches it. However, as MTB evolves by deletion, ancient DNA may not have all the plasmids of a modern strain and the match may be incomplete. "The fascinating thing is that the Vács mummies are almost unique," says

Donoghue. "We've got mummified lungs, we've got bones, we've got calcified pleura and the preservation is good enough you don't have to fish out the MTB DNA by using modern bait DNA in order to catch it."

An Old Mystery, Disputed

At the end of the 1990s, Didier Raoult, M.D., Ph.D., director of the Research Unit in Infectious and Tropical Emergent Diseases at the University of the Mediterranean in Marseille, France, tried to solve the mystery of why plague spread. "We started in Marseille with the remains of people who died of plague," says Raoult. "Marseille has been destroyed by plague several times." Raoult worked with an anthropologist and a young dentist to examine genetic material in the pulp of teeth, rather than bones, using a one-off PCR study protocol Raoult designed and dubbed "suicide PCR" to avoid contamination. There was a question of whether amplifying DNA after hundreds of years would work. "The other question was how *Yersinia pestis* caused this epidemic that was so rapid at a time when there were very few rats in Northern Europe," says Raoult.

The popular theory is that plague was transmitted by fleas from infected rats that then bit humans, but Raoult didn't think this could explain the speed of plague dissemination that killed 60% of the population. "Specifically, because rat fleas don't like to bite humans," says Raoult. Along with his colleague, Dr. Michel Drancourt, Raoult consulted papers from French investigators written in the 1920s and 30s which documented that the bacillus could survive in soil; the papers also noted that *Y. pestis* was found in lice. "We speculated that the old outbreak may have been caused by rat fleas but the interhuman transmission was by the louse," says Raoult. "Because at this time lice were very, very common. Everybody got lice."

The researcher demonstrated *Y. pestis* was transmitted by lice to rabbits. They next harvested lice from people infected with plague in the Congo and found *Y. pestis* in the lice. They also knew that in any plague epidemic, people also get *Bartonella quintana*, trench fever. They knew there was an association between trench fever and *B. quintana* from prior work looking for louse-borne infections in the tooth pulp of soldiers of Napoleon's army." We tested also plague foci to see if they could get concomitant infections between *B. quintana* and plague, "says Raoult. "It means when you get the outbreak of *Yersenia pestis*, you also get the outbreak of *Bartonella quintana*." They tested 2000 pieces of the soldiers' uniforms with mummified lice and found a probable match. "We found that based on these three evidences, the pandemic had been caused by the transmission of plague by lice," says Raoult.

Checking for Suspicious Deposits

Arteriosclerosis has replaced infectious diseases as the top killer in the developed world. It is commonplace in modern humans, and found even in younger, asymptomatic people. Vascular calcifications in mummies that pointed to arteriosclerosis were observed over a century ago, as well as in recent CT mummy scans.

Between 2008 and 2012, the HORUS Study team, a multinational collaboration, conducted a series of CT scans on 137 adult mummies from four locations—Egypt, Alaska, Peru and southwestern U.S. —ranging from circa 3100 BCE to the early 20th century. Some mummies were man made and some were natural; their ages dated within a variance of 10 years. Calcium deposits indicating probable or definite arteriosclerosis was found in the carotid, iliac, and femoral arteries in 47 mummies. The older the mummies were, the more deposits were found. The mean age of death for mummies with arteriosclerosis was 42 years old with arteriosclerosis in one or two locations, 44 years old with arteriosclerosis in three to five locations, and 32 years for those without it.

Study collaborator Gregory Thomas, M.D., M.P.H., medical director of the MemorialCare Heart and Vascular Institute in Long Beach, California, remembers imaging the mummy of Ahmose-Meritamun, an Egyptian princess who lived between 1580 and 1550 BCE. "It was just striking to think that someone had coronary artery disease in 3550 BCE," says Thomas. "It struck me that we were too smug in what we cardiologists believe are the causes of arteriosclerosis." The researchers concluded that arteriosclerosis was probably part of the natural human aging process.

According to Thomas, inflammation alone would not explain the appearance of calcium in the arteries of a high percentage of mummies in the same spots in which calcium appears in modern humans. "It is hard to fathom it is anything but atherosclerosis," says Thomas. He notes that modern-day patients with systemic inflammation such as lupus or rheumatoid arthritis develop arteriosclerosis 10 to 20 years earlier than expected. Chronic infections including parasites may well have fanned the inflammation.

"I think that the research emphasizes that we don't understand the cause of those diseases as much as we used to think we did," says Randall Thompson, M.D., of St. Luke's Mid America Heart Institute and lead author of the study. "Traditional effects only explain half the risk factors, if you look at epidemiological studies or predictive models. It makes us wonder if we are missing a risk factor, or should look for other causes beyond what we know."

Dr. Frank Rühli, professor and head of the Center for Evolutionary Medicine at the University of Zurich and co-head of the Swiss Mummy Project, was involved in imaging several high-profile Egyptian mummies. He also participated in the imaging of "Otzi the Iceman," a 3500-year-old mummy found in Italy who was found to have died from an arrow. "From a medical perspective, what is interesting is that the Iceman already had arteriosclerosis," says Ruhli. "A lot of diseases we attribute to modern times are probably more ancient than we think."

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